THE DEVELOPMENT OF PHYSIOLOGICAL RACES OF *PUCCINIA GRAMINIS TRITICI* SINGLY AND IN ASSOCIATION WITH OTHERS.

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(Seventeen Text-figures.)

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Introduction.

In cereal growing areas where stem rust of wheat is prevalent, it is customary to aim to control the disease by the use of resistant varieties. In the breeding of such rust resistant varieties, selections are usually made during the segregating generations under rust epiphytotic conditions in the field. The hybrid progenies are grown in close proximity to spreader rows of rust susceptible varieties which, early in the season, are inoculated with a collection of physiological races of *Puccinia graminis Tritici* E. and H. in the hope that most of them will develop and contribute their share of the rust inoculum present. Some of the races are invariably less virulent than others and little is known of their fate or of their usefulness in such field studies. This study was initiated to determine the fate of the weaker races when grown in competition with others on adult plants in the field and on seedlings in the greenhouse.

Review of Literature.

Recently, excellent reviews have been published dealing with the competition and association effects of fungi (D'Aeth, 1939; Porter and Carter, 1938; Waksman, 1937). These cover the work that has been done on association between different species of fungi as well as those concerned with the growth of isolates within a species. For this reason, it is proposed to review only those papers considered relevant to the material presented in this study.

Schmitz (1925), in studying *Fomes pinicola* Fries, paired four cultures from four hosts: Douglas fir, white fir, western hemlock and western white pine. He found that the hyphae of isolates from one host would not intermingle with those from another.

Cayley (1923, 1931), in her work with *Diaporthe perniciosa* Marchal, showed that single spore isolates from the same strain would mix, but if isolates of unlike strains were brought together, each appeared to inhibit the growth of the other. Widely different hosts did not necessarily yield strains that would not mix, but averting strains were obtained from different perithecia located on the same plant.

Nakata (1925) found that when isolates of different strains of *Sclerotium rolfsii* Sacc. were paired, there was either mutual or one-sided aversion. It was thought that the antagonism was due to a substance secreted by the mycelium of the fungus independent of the medium.

Vandendries (1932, 1933), Vandendries and Brodie (1933) and Brodie (1935) have studied the association of isolates of the same species, particularly in connection with the phenomenon of aversion and with sex. Similarly Kaufert (1936), working with *Pleurotus corticatus* Fries, mated haploid lines and found a complete range of reaction between isolates. Some were compatible when paired, others were antagonistic.

Hoppe (1936) presented evidence of aversion between isolates of *Diplodia zeae* (Sch.) Lév. He determined the distribution of strains of this organism on the basis of their aversion towards each other. Twenty-five cultures were obtained from widely separated regions in the corn belt of the United States, and only two of these proved to be of the same strain. Three of these strains, Nos. 26, 73 and 150, isolated from rotted kernels in different regions, were inoculated hypodermically into ears of corn. Isolations were made from kernels picked at random from the diseased ears and their identity was established by the aversion test. A sequence of inhibiting effects occurred between the strains; No. 73 inhibited No. 150, while No. 26 inhibited both Nos. 73 and 150. Hoppe believed aversion could explain his results, but suggested that differences in rate of spore germination and subsequent growth may be associated with the inhibiting powers of some strains of *Diplodia zeae.*

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Christensen and Davies (1940), in studying the effects of bacterial-staled agar on the variability of *Helminthosporium sativum* P.K. and B, race 1, found that some variants grew faster than the parent culture and were thus most easily isolated when both variant and parent were growing together in the same flask. In this way, they demonstrated that if a stock culture of *H. sativum* is placed on a bacterial-staled agar, it may be lost, since variants more tolerant to that medium can arise and completely overgrow the parent culture.

Stakman, Cassell and Loegering (1940) have reported marked population shifts in physiological races of *Puccinia graminis Tritici*, when sampling was done throughout the United States over a period of ten years. Race 56 constituted 0·3 per cent. of all isolates in 1930 and by 1939 this figure had increased to 66 per cent. Other races showed a decline in the same period, and it was stated that temperature and other meteorologic factors may influence these shifts.

Similar changes in the rust flora have been shown by Newton (1938) for Canada and by Waterhouse for Australia. In the latter country Waterhouse (1938, 1939) has identified races of *Puccinia graminis Tritici* over a period of seventeen years. Race 34 first occurred in November, 1925, and within two years it had virtually excluded the other six races previously present. The predominating effect of this race has been explained on two bases. First, it has a wider host range than the other Australian races, and secondly, new crops of uredospores are produced more rapidly, and in this way it is more virulent.

These data collected on stem rust are typical of those reported by many workers in different countries covering long periods of time. In a single growing season, changes in the rust flora may occur, and when many races are used to create an epidemic in the field, some predominate after several weeks (Watson, 1941).

Cultural Methods in the Greenhouse.

Cultures of *Puccinia graminis Tritici* were obtained from E. C. Stakman and W. Q. Loegering and represented random collections of five common races with numbers 17, 19, 34, 56 and 147. The cultures were started from single pustules and not single spores, so that several biotypes may have been present in each collection used in this study. The spores from single pustules of each race were increased on Little Club and after one generation, all five races were cultured on two varieties, Little Club and Soft Federation, for six uredospore generations at two different average temperatures of 73° and 80°F. Differential varieties were inoculated periodically to check the purity of each culture. After the first generation on Little Club uredospore mixtures of known concentrations were prepared by the use of the 'Improved Neubauer Counting Chambers' and by inoculating spore suspensions onto differential varieties. These mixtures were of races 19 and 34, 19 and 56, 34 and 56, and 19, 34 and 56. Spores from each mixture were inoculated onto seedling leaves of four pots of each of the two above-mentioned varieties. Two pots of Little Club and the same of Soft Federation were then placed in greenhouses at average temperatures of 73° and 80°F. In this way, there were two replications for each variety at each temperature.

After 10–11 days, 6–10 uniformly infected plants of Little Club and Soft Federation were used to inoculate these same varieties, in addition to varieties that differentiated between the races of each particular mixture. For example, races 19 and 34 on Little Club were transferred to two pots of Einkorn and to one pot of Little Club, the latter thus serving to perpetuate the mixture. Inoculum of these same races from Soft Federation was used to inoculate Soft Federation and Einkorn.

Stakman, Popham and Cassell (1940) already have pointed out that on one variety such as Marquis, the ratio between type 2 and type 4 uredosori can be used to estimate the percentage of races 38 and 17 in a mixture. The method suggested by them has been used throughout this study. On Einkorn, race 19 causes a type 3 pustule[*] and race 34 causes only flecks and type 1 pustules. The aggregate number of each type of uredosorus found on a total of from 40–60 leaves, was used to estimate the percentage of these two races in a mixture. Exactly the same technique was used with mixtures of races 56 and 19, since races 56 and 34 give the same reaction on Einkorn. When a mixture of races 34 and 56 was analysed, Arnautka was chosen as the differential variety. The latter shows sharp flecks when inoculated with race 56, while typical type 3 and type 4 pustules are produced when the inoculum is of race 34.

Since race 19 can be estimated on Einkorn, and since Arnautka differentiates races 34 and 56, it is possible to analyse a population of races 19, 34 and 56 by inoculating simultaneously with this mixture, seedlings of Arnautka and Einkorn. The type 3 pustules on Einkorn indicate the percentage of race 19, the flecks and type 1 pustules the combined percentage of races 56 and 34. The flecks on Arnautka indicate race 56 and the type 3 pustules races 19 and 34. Since the percentages of races 19 and 56 can be obtained directly, the percentage of race 34 is calculated by difference.

During the course of the experiment it became possible to study the effect of association of races at an average temperature of 89°F. Various combinations of pairs of races and one of four races were grown on either Little Club or Soft Federation or both. One mixture involving the races 17, 19, 34, 56 and 147 was grown for five generations with five replications on three varieties, Little Club, Soft Federation and Thatcher, at this higher temperature.

TABLE 1.

Pathogenicity of Five Physiological Races of Puccinia graminis Tritici *expressed in Means of Infection produced on Five Differential Varieties.*

Mean reaction of differential varieties.					
Race No.	Little Club.	Marquis.	Arnautka.	Einkorn.	Vernal.
17	4	4−	4=	3	1=
19	4	2−	4=	3	0;
34	4+	4−	4	1=	0;
56	4	3+	1=	1=	1=
147	4++	4+	1=	1=	4±

The pathogenicity of these five races is given in Table 1, which represents mean reaction types taken from the published work of Stakman and Levine (1938). From the table, it can be seen that Marquis, Arnautka, Einkorn and Vernal will together serve to estimate the components of the mixture, according to the following scheme:

Marquis: Type 2 pustules indicate race 19. Type 3 and type 4 pustules indicate races 17, 34, 56 and 147.

Arnautka: Flecks indicate races 56 and 147. Type 3 and type 4 pustules indicate races 17, 19 and 34.

Einkorn: Flecks indicate races 34, 56 and 147 and type 3 pustules indicate races 17 and 19.

Vernal: Flecks indicate races 17, 19, 34 and 56 and type 3 pustules indicate race 147.

It is possible, either directly or indirectly, to obtain the percentage of any one race in this mixture by counting the total number of infections of both types on 40–60 leaves of each of the four varieties.

EXPERIMENTAL RESULTS.

(*a*). *Races grown Singly in the Greenhouse.*

Methods that were used in this study are possible if all four races, when inoculated singly onto these differential varieties, cause approximately the same number of infections per leaf. If race 56 causes just twice as many flecks on Vernal as it causes type 3 pustules on Marquis, then race 147 must cause double the number of type 3 pustules on Vernal as it causes type 3 pustules on Marquis. If a significant interaction exists between race and variety, a correction factor must be established to estimate the percentage.

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[*] Infection types are those used by E. C. Stakman and M. N. Levine, *Minn. Exp. Stat. Tech. Bull.*, 8, 1922.

The extent of this differential response, if any, was studied by inoculating seedlings of the four differential varieties with each of the five races singly. Spores from 6–10 uniformly infected leaves of Little Club were brushed onto seedling leaves of Marquis, Arnautka, Vernal, Einkorn and Little Club and all were incubated in the same chamber. After 10–14 days, depending on weather conditions, the number of infections on 27 leaves of each variety except Little Club was counted and the aggregate for each pot determined. Replication was obtained by inoculating these seedlings in four of the six generations through which each race was carried on Little Club. In this way, there were 108 leaves used to calculate a mean for the infection of any variety by a particular race. The mean for each variety and race is given in Table 2, and, from the figures presented, it is clear that considerable variation occurred.

TABLE 2.

Average Number of Infections on Seedling Leaves of Four Varieties of Wheat when inoculated with Five Races of Puccinia graminis Tritici.

Race No.	Variety and Number of Infections per Leaf.				Mean Number of Infections on 27 Leaves.
	Marquis.	Arnautka.	Einkorn.	Vernal.	
17	5·3	6·2	7·9	5·7	166·8
19	12·3	10·3	9·8	13·1	307·6
34	8·9	8·1	8·6	9·7	238·3
56	9·3	9·1	9·8	15·0	292·3
147	6·9	9·5	7·7	5·3	198·1
	Mean Number of Infections on 27 Leaves.				
All Races	228·1	233·6	236·5	264·9	

S.E. of difference between two means = ±38·43.

Contributing factors may have been variation in the amount of inoculum used in the replications, as well as differences in temperature and light conditions. The analysis of variance given in Table 3, indicated highly significant F values for races and replications. Races 17 and 147 caused less infection centres on all varieties than did races 19 and 56, but not significantly less than race 34. The infection caused by race 34 was less, but not significantly less than races 19 and 56.

So far as the technique used in this experiment is concerned, it is important that no significant interaction between races and varieties was found, but the highly significant differences found between races is difficult to explain. It has been the writer's experience that isolates of race 17 show a pronounced ability to form flecks on resistant varieties and type 3 or type 4 pustules on varieties susceptible to them. Those isolates, however, give no visible indication of infection on varieties having the Kanred type of resistance. It cannot be assumed that the race 17 used in this study would give a reaction on the four varieties tested in any way similar to that given on Kanred, and yet the analysis shows that both race 17 and race 147 caused significantly less infections on these four varieties than did races 19 and 56.

Spore germination tests were not made on the races at the time of inoculations. All seedlings were inoculated within an hour of one another on the same day, and the spores were always approximately of the same age. Since spores of all races were still attached to the mycelium within the pustule, they were assumed to be equally viable. If the races show very great differences in spore germinations at different temperatures, as Cassell (1938) has found, the results could be partly explained. No data were obtained on the effect of temperature on the germination of uredospores of these races. It will be mentioned later that in studies of association, race 147 was never able to maintain

TABLE 3.

Analysis of Variance of Pustules per Pot of Four Varieties of Wheat tested with Five Races of Puccinia graminis Tritici.

Variation due to	D.F.	Mean Square.	Standard Deviation.	F.
Replications	3	83,343·216		5·639[1]
Races	4	57,596·518		3·897[1]
Varieties	3	5,416·183		0·367
Races × varieties	12	9,601·111		0·650
Error	57	14,779·445	121·5707	
Total	79			

[1] Exceeds the 1 per cent. level of significance.

itself against other races and was virtually lost after several generations of culture on Little Club. If race 147 forms less infection centres on all varieties, including Little Club, its inability to compete against others could be easily explained, but this will be discussed later.

In the determination of the percentage of the various components present in a mixture, no adjustment was made for the result given by races 17 and 147, and the aggregate numbers of infections on all leaves in the pot were taken to estimate the percentage composition.

(*b*). *Races grown in Association in the Greenhouse.*

Races 17, 19, 34, 56 and 147 occur commonly in the United States, but nothing was known of their ability to compete with each other in mixtures. Some of the races were studied more extensively than others and an effort was made to vary the percentage composition of the original mixture. In this way the development of a race, with and without an initial advantage, was studied.

(i). *Races 19 and 56.*—A uredospore mixture of 66 per cent. of race 19 and 34 per cent. of race 56 was prepared by the methods previously outlined. The percentage of each race in the mixture, after culturing on two varieties, is given in Table 4 and Figures 1 and 2. After four generations on Little Club each race occurred in approximately the same percentage as it did in the original mixture at both temperatures. During these generations there was some fluctuation in the percentage. The mixture was not analysed after one and two generations on Little Club, but after three generations at 73°F., race 19 increased from 66 to 74 per cent. but declined to 71 per cent. after four generations. At 80°F. there was an increase after three generations to 68 per cent. and an increase after four generations to 69 per cent. It is probable that these temperatures were too similar to have any differential effect on the races.

TABLE 4.

Effect of Variety on the Percentage Composition of a Mixture of Races 19 and 56 of Puccinia graminis Tritici.

Variety.	Generation Analysed.	Percentage Composition of the Mixture.			
		73° F.		80° F.	
		Race 19.	Race 56.	Race 19.	Race 56.
Little Club.	Original.	66	34	66	34
	3	74	26	68	32
	4	71	29	69	31
Soft Federation.	Original.	66	34	66	34
	1	80	20	46	54
	2	21	79	44	56
	3	11	89	12	88
	4	3	97	3	97

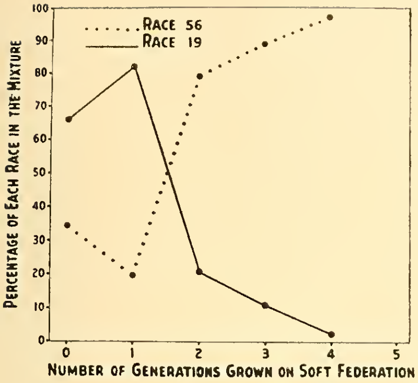

Fig. 1.

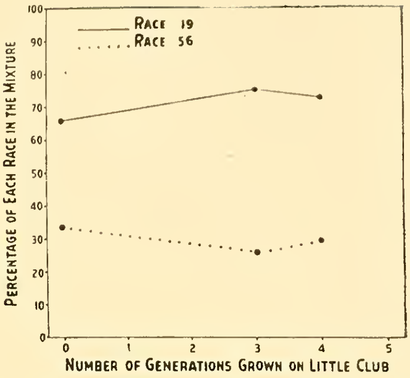

Fig. 2.

Fig. 1.—Change in per cent. of races 19 and 56 after culturing for four generations on Soft Federation at 73°F.

Fig. 2.—Effect of Little Club on a mixture of races 19 and 56 after culturing for four generations at 73°F.

When this mixture was grown on Soft Federation the situation was entirely different, as shown in Figure 1 and Table 4. At temperatures of 73° to 80°F. Soft Federation shows some chlorosis when inoculated with race 19. The same mixture of 66 per cent. of race 19 and 34 per cent. of race 56 was inoculated on to leaves of Soft Federation and at both temperatures race 56 almost completely overgrew race 19. The increase after one generation on this variety, as shown in Figure 1, cannot be explained, but the decrease for the second and subsequent generations was consistent at both temperatures. The change in population shown on Soft Federation is probably comparable to the type of interaction that occurs under field conditions when Hard Federation is used for the border rows. Soft and Hard Federation gave the same reaction when tested to this collection of race 19.

A second experiment was carried out in which race 56 was given the initial advantage as shown in Table 5 and Figure 3. Only one temperature (89°F.) was available and the mixture was cultured on Little Club. No analyses were made during the second, third and fourth generations.

TABLE 5.

Changes in Composition of a Mixture of Races 19 and 56 of Puccinia graminis Tritici *grown on Little Club at 89° F.*

Generation Analysed.	Percentage Composition of the Mixture.	
	Race 19.	Race 56.
Original	36	64
1	43	57
5	23	77

It will be noticed that at this temperature and with race 56 constituting 64 per cent. of the original mixture, there was some change in the population. These figures, in conjunction with those of Table 4, are considered to indicate how the constitution of the original mixture may influence the population after several generations of culturing on a susceptible variety. On the basis of these tests race 19 did not compete so well on Little Club at either 73° or 80°F. as did race 56 when the latter had a similar advantage and the mixture was grown at 89°F. When race 19 had the advantage, as in the first experiment, race 56 could maintain itself over a period of four generations, and so race 19 did not increase. From these results, it is unknown how race 19 would have developed at 89°F. with an original advantage of 28 per cent. It cannot be said, therefore, that race 56 is better suited to high temperatures, although it seems true that the temperature

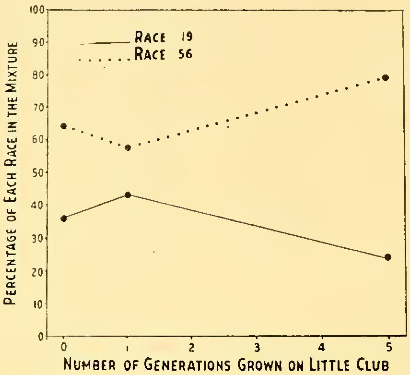

Fig. 3.—Effect of giving race 56 an initial advantage and culturing the mixture on Little Club at 89°F. for five generations.

and the amount of inoculum of each race present in the original mixture have some effect on the composition in subsequent generations.

(ii). *Races 19 and 34.*—The results obtained when races 19 and 34 were grown in association were somewhat different from those obtained with races 19 and 56. Tables 6 and 7 and Figures 4 and 5 summarize the data.

TABLE 6.

Effect of Variety on the Percentage Composition of a Mixture of Races 19 and 34 of Puccinia graminis Tritici.

Variety.	Generation Analysed.	Percentage Composition of the Mixture.			
		73° F.		80° F.	
		Race 19.	Race 34.	Race 19.	Race 34.
Little Club.	Original.	44	56	44	56
	2	44	56	46	54
	3	40	60	23	77
	4	19	81	30	70
Soft Federation.	Original.	44	56	44	56
	1	33	67	16	84
	2	19	81	17	83
	3	9	91	2	98
	4	2	98	0	100

TABLE 7.

Changes in Composition of a Mixture of Races 19 and 34 of Puccinia graminis Tritici *grown on Little Club at 89° F.*

Generation Analysed.	Percentage Composition of the Mixture.	
	Race 19.	Race 34.
Original.	51	49
1	31	69
5	19	81

At both temperatures with an initial advantage of 12 per cent., race 34 tended to overgrow race 19 when the mixture was cultured on Little Club, but for some reason the result at 80°F. was less marked than at 73°F. On Soft Federation at both temperatures, the shifts were definite and similar to those already described when races 19 and 56 were cultured on this variety. At 80°F. the mixture was entirely of race 34 after four generations. When a mixture of 51 per cent. of race 19 and 49 per cent. of race 34 was

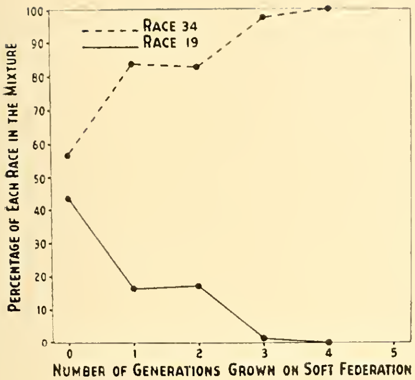

Fig. 4.

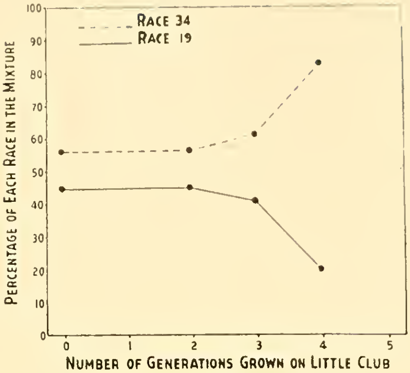

Fig. 5.

Fig. 4.—Change in population when races 19 and 34 were cultured on Soft Federation for four generations at 80°F.

Fig. 5.—Change in population after growing races 19 and 34 on Little Club for four generations at 73°F.

cultured for five generations on Little Club at 89°F., as shown in Table 7 and Figure 6, there was a very marked change in population, and race 19 declined to 19 per cent. over this period. Race 34 competed very effectively with race 19 under the conditions of the experiment and dominated the population, whether it had a substantial advantage at the outset, or whether both were started in about equal amounts.

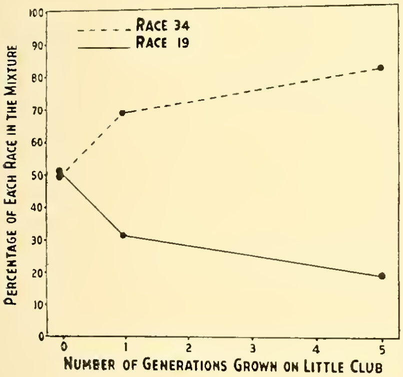

Fig. 6.

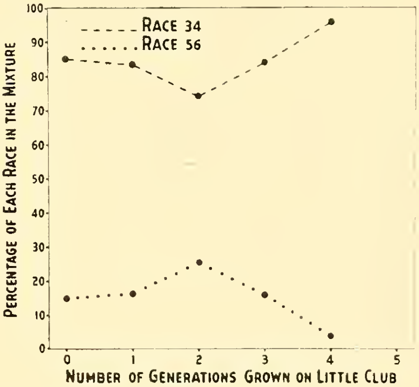

Fig. 7.

Fig. 6.—Change in population after growing races 19 and 34 on Little Club for five generations at 89°F.

Fig. 7.—Result of growing races 34 and 56 on Little Club for four generations at 73°F.

(iii). *Races 34 and 56.*—These two races are virulent on both Little Club and Soft Federation and the changes in populations are given in Tables 8 and 9 and Figures 7, 8 and 9.

At temperatures of 73° and 80°F. the results are not very definite. A mixture of 85 per cent. race 34, grown for four generations at 73° and 80°F. on Little Club, changed to 95 and 96 per cent. respectively. On Soft Federation, however, race 56 increased slightly to constitute 19 per cent. of the mixture at the end of this period. There was considerable fluctuation when these races were grown at the temperatures mentioned, irrespective of whether they were cultured on Little Club or Soft Federation.

When races 34 and 56 were mixed in approximately equal amounts, as indicated in Table 9, and grown at 89°F., race 34 overgrew race 56 on both Soft Federation and Little Club and the latter race decreased from 45 per cent. to approximately 20 per cent. over a period of five generations. The results with this mixture were somewhat the same as those obtained on Little Club at the two lower temperatures given in Table 8, but were much more definite. This demonstrated the ability of one race to compete against another when both are growing on host tissue that at ordinary temperatures appears to be equally congenial to them.

TABLE 8.

Effect of Variety on the Percentage Composition of a Mixture of Races 34 and 56 of Puccinia graminis Tritici.

Variety.	Generation Analysed.	Percentage Composition of the Mixture.			
		73° F.		80° F.	
		Race 34.	Race 56.	Race 34.	Race 56.
Little Club.	Original.	85	15	85	15
	1	80	20	84	16
	2	76	24	75	25
	3	89	11	84	16
	4	95	5	96	4
Soft Federation.	Original.	85	15	85	15
	1	86	14	91	9
	2	82	18	89	11
	3	75	25	78	22
	4	84	16	81	19

TABLE 9.

Effect of Variety on the Percentage Composition of a Mixture of Races 34 and 56 of Puccinia graminis Tritici *grown at 89° F.*

Variety.	Generation Analysed.	Percentage Composition of the Mixture.	
		Race 34.	Race 56.
Little Club.	Original.	55	45
	1	62	38
	5	80	20
Soft Federation.	Original.	55	45
	1	61	39
	5	79	21

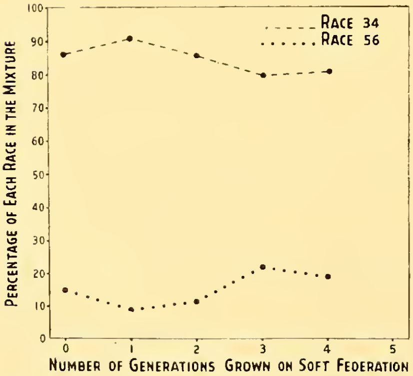

Fig. 8.

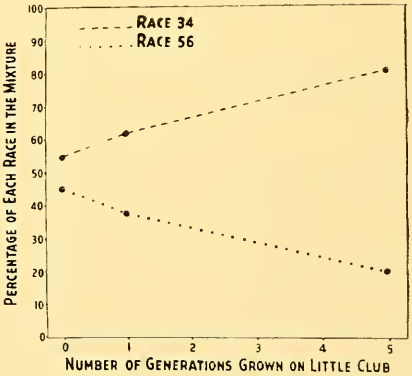

Fig. 9.

Fig. 8.—Result of growing races 34 and 56 on Soft Federation for four generations at 80°F.

Fig. 9.—Change in population after growing races 34 and 56 for five generations on Little Club at 89°F.

The probable reasons why competition occurs between these races will be discussed more fully later, but one fact should be mentioned here. At a temperature of approximately 73°F., it has been observed that all races, if grown singly on Little Club, show flecks at approximately the same time—6 to 6½ days—after inoculation, if the light intensity is high. On Little Club the epidermis was repeatedly broken earlier by uredosori of race 34 than it was by those of other races. No difference could be observed on Soft Federation for races 17, 19, 34, 56 and 147, and on Little Club there was no apparent difference between the development of races 17, 19, 56 and 147. At the higher temperatures, the more rapid development of race 34 was accentuated.

(iv). *Races 19, 34 and 56.*—This mixture was grown at 73°F. for four generations and on Little Club the results are not very conclusive, as shown in Table 10 and Figure 10.

Table 10.

Effect of Variety on the Percentage Composition of a Mixture of Races 19, 34 and 56 of Puccinia graminis Tritici *grown at 73° F.*

Variety.	Generation Analysed.	Percentage Composition of the Mixture.		
		Race 19.	Race 34.	Race 56.
Little Club.	Original.	65	23	12
	4	54	40	6
Soft Federation.	Original.	65	23	12
	4	14	62	24

Race 19 declined from 65 to 54 per cent. and race 34 increased from 23 to 40 per cent. On Soft Federation, as shown in Figure 11, the change was more marked. At 73°F., since this variety has a screening effect on race 19, the population would be expected to change. Race 19 dropped from 65 to 14 per cent. after four generations of culturing and races 34 and 56 both increased. The decrease in per cent. of race 56 on Little Club and the increase on Soft Federation is of some interest. It does not necessarily indicate that race 56 grows better on Soft Federation than on Little Club, although there was some evidence for this when races 34 and 56 were paired on these two varieties. Since no difference in rate of development was observed when these two races were grown singly on Soft Federation, it does appear that this may be a better variety on which to compare them.

(v). *Races 17, 19, 34 and 56.*—Race 56 was found to be relatively weak in its development when associated with race 34, but it was not known how other races may disturb the interaction. A mixture of uredospores was prepared in which race 34 was

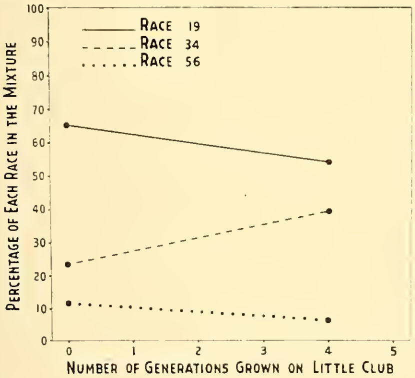

Fig. 10.

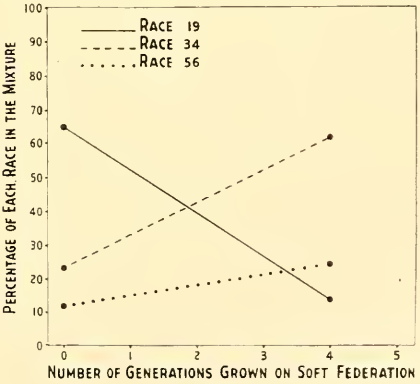

Fig. 11.

Fig. 10.—Increase in race 34 after growing a mixture of races 19, 34 and 56 on Little Club for four generations at 73°F.

Fig. 11.—Decrease in race 19 after growing a mixture of races 17, 19 and 56 on Soft Federation for four generations at 73°F.

given an advantage, races 19 and 56 were made approximately equal in amount and race 17 was used to make up 7 per cent., as shown in Table 11.

This mixture was grown at 89°F., and no matter whether it was cultured on Little Club or on Soft Federation, there was a change after five generations in the percentage of each race in the mixture. Race 34 predominated at the end of five generations, races 19 and 56 had both decreased and race 17 maintained itself with a slight increase in both cases. The decrease shown by race 19 on Soft Federation was more marked than it was on Little Club. The amount of chlorosis on the former when inoculated with race 19 at 89°F. is considerably less than when inoculated with this same race at 73° and 80°F. In addition the pustules are larger at the high temperature. For this reason, race 19 persisted longer in the mixture than it would have done at the lower temperatures.

TABLE 11.

Effect of Variety on the Percentage Composition of a Mixture of Races 17, 19, 34 and 56 of Puccinia graminis Tritici *grown at 89° F.*

Variety.	Generation Analysed.	Percentage Composition of Mixture.			
		Race 17.	Race 19.	Race 34.	Race 56.
Little Club.	Original.	7	28	42	23
	2	19	14	51	16
	5	13	15	64	8
Soft Federation.	Original.	7	28	42	23
	1	5	18	65	12
	5	12	6	76	6

(vi). *Races 17, 19, 34, 56 and 147.*—Mixtures in which these five races were associated were grown on three varieties, Soft Federation, Little Club and Thatcher, at 89°F. The original composition was determined by inoculation on to differential varieties according to the technique already described. Five replications were used in all cases. One mixture of 48 per cent. of race 17, 8 per cent. of race 19, 9 per cent. of race 34, 9 per cent. of race 56 and 26 per cent. of race 147 was grown for five generations on Little Club and Soft Federation and the results are given in Table 12 and Figure 12.

TABLE 12.

Effect of Variety on the Percentage Composition of a Mixture of Races 17, 19, 34, 56 and 147 of Puccinia graminis Tritici *grown at 89° F.*

Variety.	Generation Analysed.	Percentage Composition of the Mixture.				
		Race Number.				
		17	19	34	56	147
Little Club.	Original.	48	8	9	9	26
	5	20	42	31	6	1
Soft Federation.	Original.	48	8	9	9	26
	5	40	17	36	1	6

On Little Club race 17 declined to 20 per cent. after five generations, while race 19 and race 34 increased to 42 and 31 per cent. respectively. Race 56, although it was started in approximately the same amount as race 34 and 19, did not increase in this mixture. Race 147 was virtually eliminated at the end of five generations, even though it constituted 26 per cent. of the mixture. On Soft Federation, race 17 remained fairly constant, the figures indicating only a slight decline; race 19 increased to only 17 per cent. as compared with 42 per cent. on Little Club. This is as expected, since Soft Federation is a less congenial host for race 19 than is Little Club. Races 56 and 147 both declined in percentage. Although the results obtained in this study were consistent in all replications, it is difficult to explain why race 19 predominated in the mixture grown on Little Club. Further work is necessary to demonstrate whether a race reacts in the same way towards a second race, regardless of the presence of a third race in the mixture.

The screening effect of a variety was found to be most marked when seedlings of Thatcher were used to culture a mixture of five races over a period of four generations as shown in Table 13 and Figure 13.

TABLE 13.

Change in the Percentage Composition of a Mixture of Races 17, 19, 34, 56 and 147 of Puccinia graminis Tritici *after growing on Thatcher at 89° F.*

Generation Analysed.	Percentage Composition of the Mixture.				
	Race Number.				
	17	19	34	56	147
Original.	57	7	10	12	14
1	0	0	90	1	9
4	0	0	100	0	0

On this variety, however, nothing unexpected occurred. After one generation, races 17 and 19 were eliminated. Kanred, one of the parents of Thatcher, is immune to these two races and the latter reacts somewhat similarly to Kanred when tested with a number of races, including 17 and 19, so that exclusion after one generation would be expected, since neither race can maintain itself singly on this variety. Race 34 increased from 10 per cent. to 90 per cent. after one generation and races 56 and 147 both decreased. At the temperature of the experiment, race 34 gave an X+ reaction on Thatcher and races 56 and 147 each produced an X. On the basis of virulence alone the rapid increase of race 34 can be readily explained.

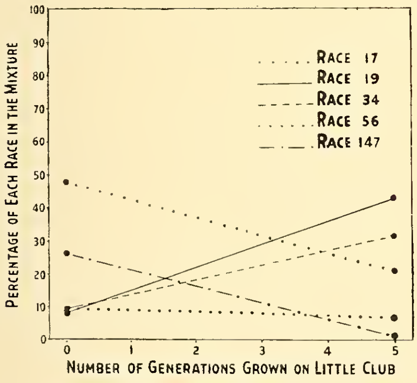

Fig. 12.

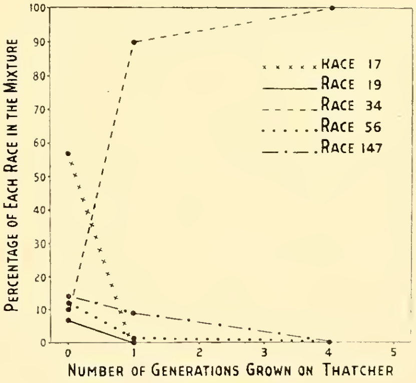

Fig. 13.

Fig. 12.—Change in population after culturing races 17, 19, 34, 56 and 147 for five generations on Little Club at 89°F.

Fig. 13.—Change in population of a mixture of races 17, 19, 34, 56 and 147 after growing for four generations on Thatcher at 89°F.

It has been mentioned previously that pustules of race 34 were observed to break through the epidermis of seedling leaves of Little Club earlier than those of the other four races. In the field, this is a most important factor to consider when determining the prevalence of races.

An experiment was set up to determine the effect of transferring inoculum to fresh seedlings after 8 days instead of after 10–11 days, as had been done in the previous experiments. In Table 14 and in Figures 14 and 15 the results of culturing in this way are given.

TABLE 14.

Effect of Variety and Frequent Transferring on the Percentage Composition of a Mixture of Five Races of Puccinia graminis Tritici *grown at 89° F.*

Variety.	Generations Analysed.	Percentage Composition of the Mixture.				
		Race Number.				
		17	19	34	56	147
Little Club.	Original.	18	44	7	4	27
	5	14	29	48	6	3
Soft Federation.	Original.	18	44	7	4	27
	5	3	66	27	3	1

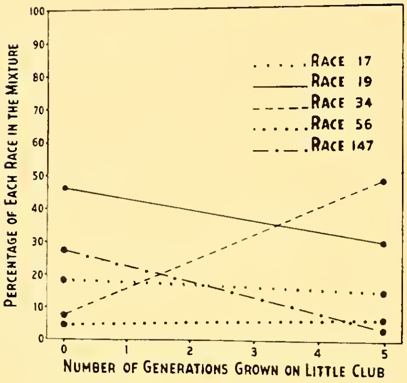

Fig. 14.

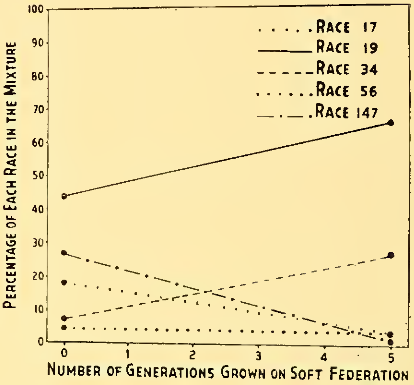

Fig. 15.

Fig. 14.—Effect of the variety Little Club and frequent transferring on the composition of a mixture of five races grown at 89°F.

Fig. 15.—Effect of the variety Soft Federation and frequent transferring on the composition of a mixture of five races grown at 89°F.

In contrast to the earlier experiments, race 17 decreased but slightly on Little Club, but constituted only 3 per cent. of the mixture when the latter was grown on Soft Federation for five generations. Race 19 behaved rather unexpectedly. At a temperature of 89°F. this race increased in per cent. on Soft Federation, but decreased on Little Club. There seems to be a possible explanation for this result. It has been stated earlier that temperatures of 89°F. increase the size of the pustules and decrease the amount of chlorosis formed, when race 19 is inoculated on to seedlings of Soft Federation. Since race 34 was not observed to develop more rapidly on Federation than other races, it would be possible for race 19 to retain its initial advantage. On Little Club, however, race 34 developed more rapidly than race 19 at temperatures of 73° and 80°F. and the difference was accentuated at 89°F. Race 34 under these conditions would be able to overcome, partly at least, the initial advantage held by race 19. Race 17 showed no tendency to dominate the mixture and race 147 decreased in per cent. until it was almost eliminated.

(c). *Races Associated in the Field.*

In the years 1939 and 1940 stem rust epidemics were induced in the field by the usual methods. Border rows of Marquis and Hard Federation were sown in 1939, and in 1940, these rows were of Hard and Soft Federation. Thirty-two races were used in the first year and 30 in the second, and their numbers are given in Figures 16 and 17.

The races were grown on seedlings of Little Club in the greenhouse and a suspension of uredospores was hypodermically inoculated into the young stems of the border row plants. From then on the methods used were somewhat different for the two years.

In 1939, single pustules were taken from the field at random from borders and hybrid rows over a period from 8th July to 26th July. In some cases the uredosori had just broken the epidermis; in others they were up to 0·25 cm. in length. No special effort was made to isolate at different times during the season. A total of 103 single pustule collections was made and the races identified in the greenhouse during 1939. In the 1940 season, pustules were collected at three different periods. The first collection was made between 18th June and 27th June, the second from 13th July to 15th July, and the third from 30th July to 3rd August. Usually between 40 and 50 samples were taken at each time, and in all 136 collections were identified in 1940. Among the 43 samples taken during the final period, 22 were of mass uredosori from the stems. When these were inoculated on to Little Club in the greenhouse, seedling leaves were so badly injured by *Helminthosporium* sp. that only one, two, or at the most three pustules developed on seedlings in each pot. Identifications of races in these collections would probably have necessitated many separations had this injury not occurred. As it happened, no more than three races were isolated from any one collection of this group.

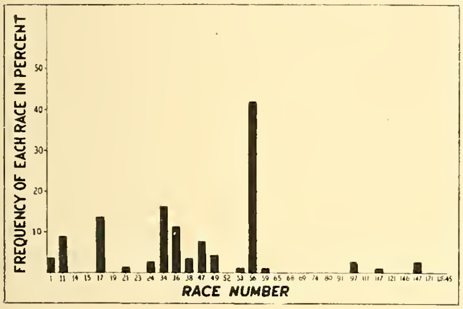

Fig. 16.

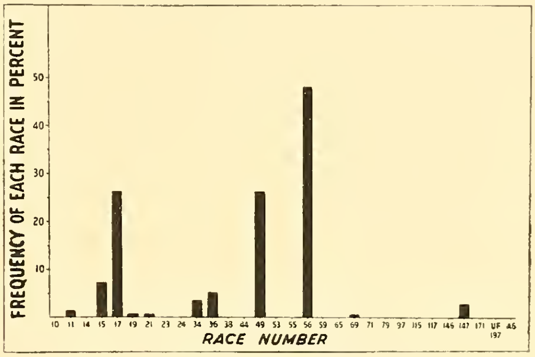

Fig. 17.

Fig. 16.—Races used in the nursery and the frequency of their recovery in per cent. in 1939.
Fig. 17.—Races used in the nursery and the frequency of their recovery in per cent. in 1940.

In both years, after inoculation of the single pustule material on to Little Club, a cellophane protecting screen was placed around each pot during the incubation period. When uredosori had developed sufficiently, inoculum was transferred to a set of differential varieties and the race of rust determined by use of the key prepared by Stakman and Levine (1938). When more than one race occurred, separations were made in the usual way.

The relative prevalence of each of the re-isolated races is given in Table 15 and in Figures 16 and 17. Race 139 was recovered from the field in 1939, but it was not used in the hypodermic inoculations. It could have been blown in from adjacent fields as uredospores, or as aecidial material from barberries. Sixteen races of the 32 used in 1939 were recovered, and of the 30 used in 1940, 11 were re-isolated. A strong criticism is that too few isolations were made to get a really random sample of the rust flora in the nursery. Despite this criticism it is significant that, in general, the same races

TABLE 15.

Relative Prevalence of Races of Puccinia graminis Tritici *in a Rust Nursery in 1939 and 1940.*

Year.	Race Number and Prevalence in Per Cent.																		
	1	11	15	17	19	21	24	34	36	38	47	49	53	56	59	69	97	117	147
1939	2·9	9·7		12·6		1·0	1·9	15·5	10·7	2·9	7·8	3·9	1·0	41·8	1·0		2·9	1·0	2·9
1940		1·5	7·4	26·5	0·7	0·7		3·7	5·2			26·5		48·5		0·7			3·0

occurred in both years, and this seems to be good evidence that when a large number of races are used to induce an epidemic, few of them really develop to epidemic proportions. Probably a better method of sampling a stem rust nursery would be to take mass collections and separate out the races in each collection. Identification of races in this study was greatly simplified since most of the samples contained only one race.

In general, single pustules yield single races, but whether they yield one or more races depends on the size of the pustule and the time of season when the sample is taken. In 1939, 75·7 per cent. of the isolates were of single races, while in 1940, omitting the 22 mass uredosori collections, 81·3 per cent. produced a single race. During this latter year, when the sampling periods were spaced at approximately two weekly intervals, the number of races isolated per pustule varied with the period. At the first period, 94·0 per cent. of the pustules yielded single races; at the second period, 79·6 per cent. were single races; but in the third period, single races were recovered from only 59·0 per cent. of the single pustules. When more than one race was present there were usually only two, although in 1939 one collection yielded three races.

The races recovered in 1939 were more uniformly distributed than in 1940, as indicated in Figures 16 and 17. It appeared that during the first season rust developed slowly, and for that reason the plot was irrigated to favour its development. No such treatment was necessary in 1940. During this latter year infections were present on the border rows before the uredosori from the primary hypodermic inoculations had broken the epidermis. Hypodermic inoculations in 1940 were made first on 17th June and on 18th June nine pustules were collected. These represented infections from spores blown from outside, and races 11, 17, 36, 49, 56 and 147 were isolated. Races 17 and 36 were mixed in one pustule and races 11 and 147 in another.

It will be seen from Figure 17 that races 17, 49 and 56 were the most common when 136 collections were grouped in 1940. It is possible that the initial advantage these races had in being present in the border rows early, may have influenced their abundance in later generations. The time of the season was found to have some effect on the prevalence of races, since race 15 was isolated twice in 94 collections made prior to 30th July, whereas it occurred eight times in 43 collections made from 30th July to 3rd August. This may have been a temperature effect, but no data were obtained on its temperature relationships.

Discussion.

Races of *Puccinia graminis Tritici* are now known to react very differently to temperature as well as to variety. There is no reason to believe that stem rust races are unlike physiological races of other fungi, which, in culture, react differently to pH, to staling substances, or to the presence of other micro-organisms. The work of Cassell (1938) has demonstrated very clearly that certain races of stem rust are aggressive at high temperatures, others thrive on their hosts at low temperatures, while still others seem to be adapted to a wide range of temperature conditions. Since there is no evidence to indicate that *P. graminis Tritici* is less variable than other fungi, it cannot be expected that the temperature relations of race 56 will be constant for all isolates that happen to give the race 56 type of infections on the differential varieties.

In the results that have been reported here, race 34 was particularly virulent on the hosts on which it was cultured. A number of other races, including races 17, 56 and 147, could develop well on the variety Soft Federation and these, in addition to race 19, caused a susceptible reaction on Little Club. When these races were mixed, however, a type of competition occurred which is difficult to explain. If any one of these races was cultured singly on 6–10 leaves of either of the two above-mentioned hosts, it could maintain itself over a number of generations and was stable in its reactions. The amount of infection did not diminish, irrespective of whether the temperature was 73°, 80° or 89°F. However, certain races always decreased in percentage when they were associated with certain other races in mixtures.

It might be expected, on the basis of the findings of Cassell, and in view of previous work by Stakman and his associates (1940), that race 56 could compete very effectively with other races when they were associated in culture at high temperatures. Such was not found to be the case. If races 56 and 34 were associated, or if they were in association with other races and the mixed culture was grown on Little Club, race 56 did not predominate over race 34 after several generations. It is possible that if race 56 had been given a big initial advantage it might have been able to overgrow race 34, but no data were obtained to support this assumption.

The amount of inoculum in the original mixture was important for some races. In most cases, race 34 overgrew other races rapidly when it was given an advantage in the original mixture. This was not true for race 17. When a mixture of 48 per cent. of race 17 and 52 per cent. of other races was cultured on Little Club, there was a decline from 48 to 20 per cent. after five generations.

It was not possible to predict the final composition of a mixture by comparing the reaction of the host when inoculated with the components singly. When race 19 was mixed with races 34 or 56 and the culture was grown on Soft Federation at temperatures of 80°F. or lower, race 19 was virtually eliminated after several generations. At higher temperatures, averaging 89°F., the result was different, not because race 19 grew better at high temperatures, but because the variety reacted differently at high temperatures to race 19. If race 19 was associated with race 34, or with race 56 on Little Club at 89°F., it was partially eliminated by both of these races.

The final composition of any one mixture after a number of generations of culturing is dependent on certain factors. First, it depends on the amount and character of each race present in the mixture. Secondly, it depends on the variety, and thirdly, the temperature insofar as it affects the fungus and the way the variety reacts to the fungus.

Some races develop well in mixtures and others do not. Race 147 belongs in the latter group. In no case was race 147 able to maintain itself after several cultural generations, despite the fact that in some of those mixtures it originally constituted up to 26 per cent. No definite evidence was obtained to account for this elimination. In the experiments made to determine the extent of interaction between the variety and the race, it was found that race 147 formed significantly less infection centres than race 56 on Marquis, Arnautka, Vernal and Einkorn. If this could be extended to include Little Club and Soft Federation, it would provide a very suitable explanation for the consistent diminution of race 147 when grown with others. Although race 147 and race 34 give identical reactions on Little Club, there is apparently a difference in their virulence, which is imperceptible to the naked eye, but which becomes important when the races are associated.

Race 34 was able to develop well in association and its ability to mature fresh crops of uredospores rapidly has already been mentioned. Other minute differences between races could be detected by suitable methods. Type 3 and type 3+ uredosori are of little use in designating infection ratings, but many hundreds or thousands of spores may be involved in such differences. No counts were made of spore production per pustule, or of percentage germination at maturity, but if these varied from race to race, competition would become evident.

Waksman (1937) has already discussed the chief factors that effect the development of any one group of micro-organisms; food supply and environmental factors have been mentioned here. To date no evidence has been obtained to indicate that one isolate of rust can inhibit or stimulate the growth of another, although the possibility of this should not be overlooked. It may so happen that some races can stimulate the growth of others by splitting complex compounds, thus making more nutrients available, as Johnston (1934) has suggested for powdery mildew and leaf rust of wheat. On the other hand there may be a genuine antibiosis, or one race may change the permeability of the cell membrane so that the relations between the host and other races in the mixture are disturbed. Probably many factors operate in this competitive effect, none of which can be stated with definiteness.

In the field experiments at St. Paul, it was found that less than half of the races used in the primary inoculation finally reached epiphytotic proportions when the border rows were of Marquis, Hard Federation and Soft Federation. Some races, either by virtue of their aggressiveness, or as a result of being blown in large quantities from adjacent rusted fields, predominated in the nursery.

Many factors are involved in this shift in population of races in the field, and the variety used as border rows contributes largely. If a uniformly susceptible variety was available a more even development of races could be expected. At present, no variety of wheat is known, which is equally susceptible to all races. Environment, too, would exert a very big influence, since rust races and varieties are known to give marked responses to temperature.

The nature of the individual race will also be important in these changes. Some races pass rapidly from the uredospore to the teleutospore stage and since the latter is non-repeating, these would have little chance of survival when grown in association with races that mature fresh crops of uredospores in quick succession and which have delayed teleutospore production. Differences in response to other organisms could also influence the survival of races. In 1939 and 1940, the bacterium pathogenic on the uredospores of rusts was present in the nursery, and although no field evidence was obtained of differential susceptibility of races to this organism, greenhouse results indicated that it probably does occur, and if so could influence the prevalence of races in the field.

From the findings outlined above, a practical question is raised as to whether large numbers of races should be used to induce stem rust epidemics in the field, or whether a few well-selected, virulent races would give better results. If physiological races are of no importance, the most convenient and effective method of obtaining an epidemic would be by the inoculation of border rows with uredospores of a virulent race adapted to a wide range of environmental conditions. In this way antagonistic relations between races, if any, would be overcome. If more were known of key races, resistance to which would automatically give resistance to other races, then single race epidemics would have much to recommend them.

It has been shown by Holton and Heald (1936) that varieties of wheat may give a reaction to single races of *Tilletia* sp. different from that given by these same varieties when tested to a composite of races. Tsiang (1940), working with *P. graminis Tritici*, found that Rival, Pilot and N-S-N-11-31-4 appeared less severely infected in a field where many races were used, than they did when exposed to epidemics of single races in isolated plots. He suggested that differential rates of spread of races in association may have been responsible for this effect. Isolation plots for testing selected resistant lines with purified races of *P. graminis Tritici* have not been used to any extent, but, in breeding for smut resistance in wheat, however, promising material is usually tested with single races (Christensen and Rodenhiser, 1940). Under certain circumstances, such a procedure for wheat stem rust may be advantageous. At St. Paul in 1939, the varieties Kenya 744 and 745 were resistant to rust (Watson, 1941). Race 15 of *P. graminis Tritici* was not isolated from the nursery in which they were grown. In the 1940 tests, Kenya 745 had up to 35 per cent. of stem rust and in three uredospore collections from a Kenya 745 derivative, race 15 was isolated. Such a change in reaction is difficult to explain. It is probably the result of a beneficial interaction between a virulent race and favourable environmental conditions.

Since the determination of the reaction of hybrids to many single races in isolated plots is difficult and impracticable under field conditions, selections can be made during epidemics caused by relatively few races, provided these races are the most virulent available, and provided they can be regarded as key races. If resistance to such key races is incorporated into new material, the latter will also possess resistance to other races. The classification of *P. graminis Tritici* so that key races are known, involves considerable work, but in Australia new lines of wheat have been developed which are known to have resistance to a large number of races of leaf and stem rust, but which were bred for resistance to only one race of each.

SUMMARY.

Four varieties of wheat, Marquis, Arnautka, Vernal and Einkorn were inoculated singly with races 17, 19, 34, 56 and 147 of *Puccinia graminis Tritici* and there were no significant interactions between variety and races, although races 17 and 147 caused significantly less infection centres than races 19 or 56. A technique was developed for the study of a competitive effect when several races of rust were associated in culture.

Race 34 always grew well and maintained itself, or increased, in percentage of the mixture when associated with one, two, three or four other races. Race 147 was always virtually eliminated from such mixtures after several uredospore generations. Temperature was found to influence the variety on which a mixture was grown and in this way to affect the final composition of the mixture. The variety Thatcher, when inoculated with a mixture of races 17, 19, 34, 56 and 147, effectively screened out four of the five races after four generations, so that there remained a pure culture of race 34. A large number of races were inoculated into susceptible border rows of a field rust nursery in 1939 and 1940, but less than half were found to reach epiphytotic proportions. Race 15 was not isolated in 1939, but was found in 1940 on F3 lines pure breeding for the Kenya 745 type of resistance.

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